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R-CHIE: a web server and R package for visualizing *cis* and *trans* RNA–RNA, RNA–DNA and DNA–DNA interactions

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ABSTRACT

Interactions between biological entities are key to understanding their potential functional roles. Three fields of research have recently made particular progress: the investigation of *trans RNA–RNA* and *RNA–DNA* transcriptome interactions and of *trans* DNA–DNA genome interactions. We now have both experimental and computational methods for examining these interactions *in vivo* and on a transcriptome- and genome-wide scale, respectively. Often, key insights can be gained by visually inspecting figures that manage to combine different sources of evidence and quantitative information. We here present R-CHIE, a web server and R package for visualizing *cis* and *trans RNA–RNA*, *RNA–DNA* and *DNA–DNA* interactions. For this, we have completely revised and significantly extended an earlier version of R-CHIE (1) which was initially introduced for visualizing RNA secondary structure features. The new R-CHIE offers a range of unique features for visualizing *cis* and *trans RNA–RNA*, *RNA–DNA* and *DNA–DNA* interactions. Particularly note-worthy features include the ability to incorporate evolutionary information, e.g. multiple-sequence alignments, to compare two alternative sets of information and to incorporate detailed, quantitative information. R-CHIE is readily available via a web server as well as a corresponding R package called R4RNA which can be used to run the software locally.

INTRODUCTION

The recent years have seen a few conceptual novelties. Since a few years, it is now possible to directly examine *cis* and *trans* RNA–RNA interactions *in vivo* and on a transcriptome-wide scale (2–5). This not only gives us a glimpse into a so-far well-concealed universe of RNA secondary structure features *in vivo*, but also enables us for the first time to probe direct *trans* RNA–RNA interactions more widely and without the need for a corresponding anchor protein or RNA. The corresponding initial protocols (2–5) still offer scope for improvement, both in terms of systematic biases and efficiency bottlenecks, but already yield transcriptome-wide evidence for direct *trans* RNA–RNA interactions that often link two or more transcripts. Raw data is typically supplied in terms of so-called duplexes, i.e. pairs of short, contiguous stretches of some-how interacting nucleotides deriving from the same or two separate transcripts, rather than in terms of direct evidence for specific base-pairs at nucleotide resolution. These raw duplexes, however, do not retain information on which biological transcript(s) they derive from. It is therefore currently largely up to the computational interpretation to convert these raw duplex data into evidence for specific *cis* or *trans* base-pairs between distinct biological entities of the transcriptome. This remains computationally and conceptually challenging (6).

Also the well-established protocols for SHAPE-like probing of RNA secondary structures in a transcriptome-wide manner *in vivo* do not yield direct evidence for specific base-pairs, but only reactivity values for individual nucleotide positions within transcripts. Any raw SHAPE data thus still needs to be processed and computationally interpreted using sophisticated methods in order to derive evidence for specific *cis* base-pairs or entire RNA secondary structures

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that could be made by a biological transcript at any specific point in time (7,8).

Overall, however, we have clearly entered the era of transcriptome-wide RNA structure and *RNA–RNA* interaction studies *in vivo* and thus require adequate tools for visualizing and interpreteting these data.

On the genomic side, the last two decades have seen an increasing range of novel methods to investigate genome interactions *in vivo*. The emerging view is that many genomes *in vivo*, including the human genome, have a distinct structural state that may change upon a changing *in vivo* environment.

Based on the original idea of chromatin conformation capture (3C) (9), the structure state of a genome can investigated on a genome-wide scale via 4C (10), 5C (11), GCC (12) and Hi-C (13–17) as well as via genome archictecture mapping (GAM) (18). While the techniques derived from 3C are based on the ligation of (mostly of pairs of) interacting DNA segments and digestion, GAM works conceptually differently and involves no ligation-step. Rather, thin two-dimensional slices of frozen cells are extracted and their single nuclear profiles extracted via laser microdissection before their individual DNA content is sequenced. By collecting data from many slices from a single frozen probe, the method ought to probe the population of cells and their nuclei at randomly distributed orientations. The underlying assumption is that pieces of genomic regions deriving from the same nuclear profile should have a higher chance of directly interacting than pieces that rarely co-segregate. Compared to 3C-derived methods, the resolution of GAM is limited by the thinness of the slices (currently around 200 nm), but has the conceptual advantage of being able to readily detect genome interactions beyond pairs of genome segments. Compared to 3C-derived methods, the raw data from GAM requires different computational analyses in order to deduce quantitative evidence for actual genome interactions.

There also exist by now a number of new studies investigating *trans RNA–DNA* interactions between the transcriptome and its genome, see e.g. (35).

Similarly to RNA structure and *trans* RNA–RNA transcriptome interactions, *trans* RNA–DNA and *DNA–DNA* interactions can be illustrated in a conceptually similar manner. In both cases, the biological entities (RNA transcripts or chromosomes) can be visualized in terms of linear lines (even if they actually correspond to circular RNAs or chromosomes). In all cases, their interactions involve one, two or several of these entities of the same (*RNA–RNA* or *DNA–DNA*) or different (*RNA–DNA*) kind. All types of interactions may comprise evidence for mutually exclusive pairwise interactions that need to be visualized. And all *cis* and *trans* features should be based on quantitative evidence (whether experimental or computational) that should be included in the corresponding figures. The main difference between the different types of *cis* and *trans* interactions is in scale and resolution, RNA structure and *trans* RNA–RNA interactions are typically studied at near-nucleotide resolution, whereas *trans* RNA–DNA and genome interactions are typically only known up to a few tens of kilobases, i.e. four to five order of magnitude difference.

While raw, large-scale data sets relating to *trans* RNA–RNA, *trans* RNA–DNA and genome interactions are typically best analysed and interpreted using computational methods utilising principled mathematical concepts in machine learning or artificial intelligence, the human brain is often still best at readily identifying relevant features and interesting patterns in figures that manage to combine different sources of quantitative evidence into one picture. This was and is our key motivation in designing R-CHIE.

There already exist a number of tools for visualizing *trans* RNA–RNA interactions, see Table 1. Likewise, there already existing several programs for visualizing *trans* DNA–DNA interactions. There is, however, not yet a tool for visualizing *trans* RNA–DNA features. Plus, all existing tools either cater for *trans* RNA–RNA or for *trans* DNA–DNA interactions, but not both.

Building on the unique features of R-CHIE's first release as a visualization tool for RNA secondary structure, we set out to significantly expand its scope to also visualize new large-scale *trans* RNA–RNA, *trans* RNA–DNA and *trans* DNA–DNA data sets. Compared to existing tools, the unique properties of R-CHIE can readily be summarized and highlighted, see Table 1 for an overview.

The new version of R-CHIE is the only tool capable of visualizing data involving multiple biological entities, e.g. multiple transcripts linked via *trans* RNA–RNA interactions, multiple chromosomes engaged in genome interactions or multiple transcripts involved in *RNA–DNA* interactions with a genome. As with RNA secondary structure features, we also expect *trans* RNA–RNA, *trans* RNA–DNA and genome interactions *in vivo* to depend on a mixture of intrinsic properties and extensic properties. Intrinsic properties, i.e. features encoded in the biological sequences themselves, may include the RNA's potential to form certain RNA structure features or the DNA's ability to engage in a range of potential genome interactions, whereas extrinsic properties may comprise proteins, ions or other additional biological entities that may also influence the formation of the actual RNA structure, *trans* RNA–RNA, *trans* RNA–DNA or genome interaction *in vivo*. In order to investigate and highlight the presence or absence of some key intrinsic properties such as the amount of evolutionary conservation, multiple-sequence alignments (MSAs) corresponding to the biological input entities can readily be integrated into the figures using R-CHIE. In the case of *cis* and *trans* RNA–RNA interactions, this includes the ability to highlight distinct evolutionary patterns such as the covariance. We have extended the popular concept of comparison plots in the original version of R-CHIE (originally called 'overlapping plots' in (1)) to *trans* RNA–RNA, *RNA–DNA* and genome interactions. Given two alternative sets of these interactions for the same set of input sequence(s), R-CHIE will first compute common and distinct features and then illustrate them seperately. Figures based on these comparison plots allow users to readily assess the sensitivity and specificity of computational predictions with respect to known features or, for example, to compare data deriving from two different experimental conditions or from two competing prediction methods.

We make R-CHIE available via several means: via an easy-to-use web-server `http://www.e-rna.org/rchie/` that generates figures on the fly and showcases several rele-

vant examples, as well as dedicated R-package R4RNA for downloading and usage on a local computer.

MATERIALS AND METHODS

RNA secondary structure features

The new version of R-CHIE continues to offer the full functionality of the initial version of R-CHIE (1) in terms of visualizing RNA secondary structure features. In particular, the six key existing types of diagrams (single structure, double structure, comparison structure, single structure covariation, double structure covariation and comparison structure co-variation) continue to be supported. They essentially allow any user to visualize and compare one or two sets of RNA structure features with or without showing one or two corresponding multiple sequence alignments. Most importantly, R-CHIE continues to offer users the possibility to visually encode quantitative information on invidual RNA structure features such as helices or individual base-pairs. Examples of colouring schemes include colouring by *P*-values (to encode estimation of the reliability of RNA structure predictions), by covariance (to encode information on the evolutionary support via a corresponding multiple-sequence alignment), by primary sequence conservation or any other user-defined quantitative scheme whose values can be assigned to individual RNA structure features.

Trans RNA–RNA interactions

A novel feature in the new release of R-CHIE is that it now supports the visualization of *trans* RNA–RNA interactions. By definition, *trans* RNA–RNA interactions involve base-pairs *between* two or more RNA sequences. As *trans* interacting RNAs may also comprise RNA structure features, i.e. *cis* base-pairs linking pairs of nucleotides within the same RNA, R-CHIE allows the joint visualization of both, *cis* and *trans* RNA–RNA interactions. In particular, users can now readily illustrate two sets of features concerning the same sequence, e.g. a set of known reference features and a set of predicted features or two alternative sets of predicted features, even if these features comprise *trans* RNA–RNA interactions or more than two interacting RNAs, see Figures 1–3.

We extended the concept of these plots to so-called comparison plots, a unique and useful feature in R-CHIE, that allows for the automatic comparison of two alternative sets of *cis* and *trans* features. For this, R-CHIE first computes common and distinct features between the two sets of information and then illustrates them separately. These comparison plots thereby enable, for example, the straightforward and intuitive assessment of the sensitivity and specificity of a prediction method, see Figure 4, or the comparison of features between two different experimental conditions. These comparison plots can either be made with all involved RNAs shown next to eachother along a single horizontal line in the manner depicted in Figure 1 or by highlighting one of several RNAs as reference by depicting it on its own as the bottom as shown in Figure 4.

Any of the types of plots for illustrating RNA structure and *trans* RNA–RNA interactions can be combined with the possibility to shown corresponding multiple-sequence alignments for each sequence. Naturally, for different input sequences, this may involve different sets of species at different evolutionary distances. Technically, R-CHIE handles these differences automatically when generating the requested figures. We decided, however, to allow the user to adjust key parameters in order to further optimise the layout of the figure, if needed.

DNA–DNA genome and *RNA–DNA* transcriptome interactions

Conceptionally, genome interactions are very similar to *cis* and *trans* RNA–RNA interactions. Both involve sequences (RNA or DNA) for now both depicted as linear sequence despite the existance of circular RNA and DNA genomes and circular RNAs. Most importantly, both involve *pair-wise* interactions (RNA–RNA, RNA–DNA or DNA–DNA) between one chunk of sequence and another chunk of sequence of the same or different type (RNA, DNA). The key difference between *cis* and *trans* RNA–RNA interactions and typical information on *RNA–DNA* and *DNA–DNA* interactions is their scale. For *cis* and *trans* RNA–RNA interactions, we typically have information on individual base-pairs at nucleotide resolution, whereas current information on *cis* and *trans DNA–DNA* and *RNA–DNA* interactions is currently typically supplied at a resolution of at most 1 kilobase (kb) (19,35), where one *contiguous sequence interval* interacts in some kind with another *contiguous sequence interval* of the same length. R-CHIE readily extends to *cis* and *trans RNA–DNA* and *DNA–DNA* interactions and now offers the ability to provide a multitude of useful types of figures, see Figures 5 and 6 for *trans* DNA–DNA interactions and Figure 7 for *trans* RNA–DNA interactions.

Table 1 provides an overview of the functionality of R-CHIE compared to existing programs for visualizing genome interactions. We here highlight only features unique to R-CHIE. Two concepts are particularly note-worthy. One is R-CHIE's ability to readily compare two set of information on genome interactions, both *trans* DNA–DNA as well as *trans* RNA–DNA interactions. These can either be illustrated in a straightforward manner, see Figure 5 or via so-called comparison plots which first compute common and distinct features and then illustrate them separately, see Figure 6. Another unique concept of R-CHIE is its ability to visualize evolutionary information alongside genome interactions. This is primarily achieved by showing corresponding multiple-sequence alignments (MSAs). By default, these MSAs are illustrated in a way which colour-codes key evolutionary features such as primary sequence conservation and gaps at nucleotide resolution within the interacting regions. In addition to this color-coding within the MSA itself, the user can choose to colour the interacting arcs according the extent and type of the underlying evolutionary support within the interacting regions of the MSA, e.g. in terms of primary sequence conservation or co-variation. For this feature to be useful in practice, however, the resolution of the experimental procedure that was employed to derive genome interaction data has to be of the same order of magnitude as the evolutionary information to be visual-

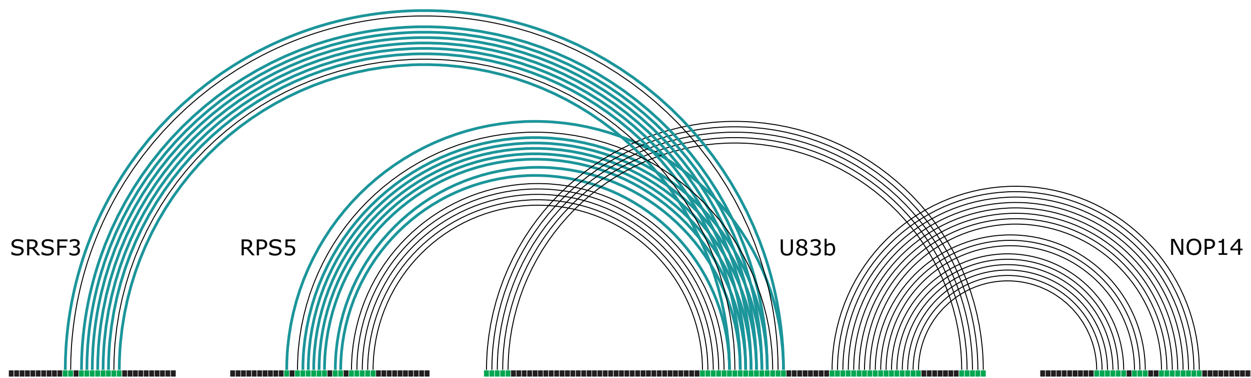

Figure 1. Figure depicting the interactions between snoRNA U83B and multiple targets (4,21,22). For the targets, we only show the sub-sequence surrounding the target side (with 10 nt on either side of the target side): SRSF3 (5210:5240), RPS5(457:493), NOP14 (1111:1150). The corresponding sequences were downloaded from ENSEMBL (22): U83B (ENSEMBL gene ID ENSG00000209480), SRSF3 (ENSG00000112081, sequence interval (5210:5240)), RPS5 (ENSG00000083845, sequence interval (457:493)), NOP14 (ENSG00000087269, sequence interval (1111:1150)). Arcs depict sequence positions interacting via a base-pairs. Arcs marked in colour highlight clashing interactions.

Figure 2. Figure showing the interactions between 18S rRNA (RFAM family RF01960, first 350 nt out of 1869 bases) and H/ACA box snoRNAs U69 (RF00265), ACA10 (RF00264) and ACA31 (RF00322). RNA structure features and sequences derived from RFAM (21), *trans* interactions extracted from SNORNABASE (24) (https://www-snorna.biotoul.fr/). Each arc depicts a pair of sequence positions interacting via a *cis* or *trans* base-pair. Arcs marked in colour highlight clashing interactions.

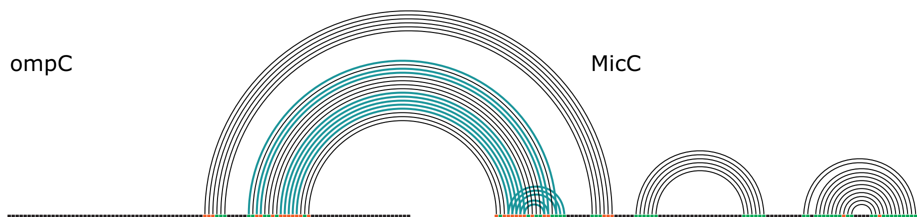

Figure 3. Figure showing the interactions between mRNA ompC (sequence interval (400, 500) out of 1800 bases, no secondary structure information available) and sRNA MicC (23). As usual, each arc depicts a pair of sequence positions interacting via a *cis* or *trans* base-pair. Arcs marked in colour highlight clashing interactions.

ized in the corresponding MSA. These two unique features can be also be combined into plots which show the same MSA in two different ways, one for each of the two corresponding sets of genomic information. In that case, each MSA illustrates the evolutionary support (or lack thereof) for each set of genomic information. In particular when combined in a comparison plot, these unique R-CHIE features can readily highlight parts of an MSA that require improvement (in terms of alignment quality) or parts of a prediction that are poorly supported by evolutionary evidence. For this, users can, for example, optionally zoom into select features that are particularly trustworthy (e.g. in terms of estimated *P*-value or experimental evidence) by imposing a corresponding quantitative threshold on the feature to be visualized, see Figure 5. In that case, users can choose to depict the select genome interactions as arcs rather than in the usual heatmap, see Figure 5. As for *trans* RNA–RNA interactions, figures illustrating genome interactions can readily handle more than two input sequences. And, as discussed before in the context of *cis* and *trans* RNA–RNA interactions (see Figure 4), it is also possible to visually highlight one of the input sequences as a reference sequence in the context of genome interactions.

The new R4RNA R package

The new version of R-CHIE now operates on R code that makes full use of the `data.table` library in R, thereby significantly increasing the computational efficiently with which operations on large set of data can be processed while simultaneously yielding more compact source code that it easier to write, read and maintain. For this, we essentially

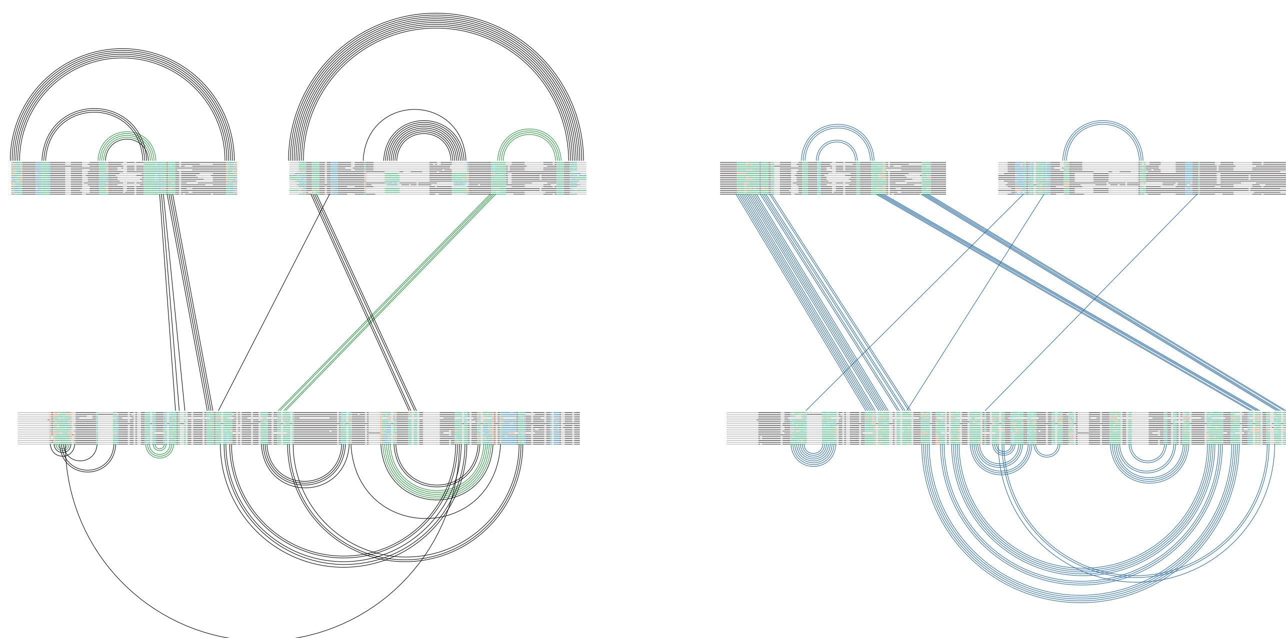

Figure 4. Comparison plot illustrating the common and distinct features of two sets of predicted *cis* and *trans* RNA–RNA interactions involving the same three non-coding RNAs as in Figure 1. Here, the 18S rRNA sequence is highlighted as reference and shown below, snR41 (left) and snR128 (right) are shown on top. The left part of the figure illustrates the sensitivity of the predictions w.r.t. the first set of predictions which serves as a reference: true positive, i.e. correctly predicted, *cis* and *trans* base-pairs are highlighted in green. False positives, i.e. known features that are missing from the prediction, are shown in black. The right part of the figure highlights the specificity of the predictions (and would be devoid of arcs and lines in case of perfect specificity). Any false positive *cis* and *trans* features are shown as blue arcs and lines, respectively. They correspond to the RNA structure and *trans* RNA–RNA interactions that are not part of the reference, but only part of the second set of predictions. The color-coding within the MSAs is according to the same legend as in Figure 1.

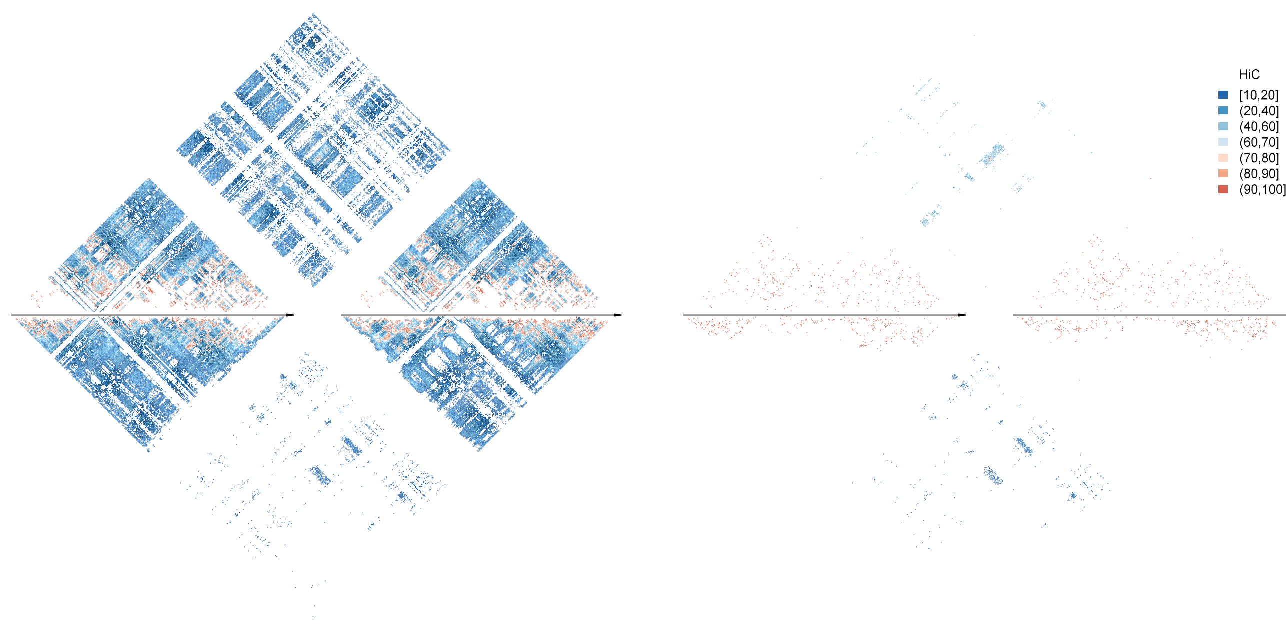

Figure 5. Plots showing two Hi-C sets for human chromosome 10 and 11 from two different experiments, data set 1 (top) (25,26) and data set 2 (bottom) (27), depicted in the usual manner via a heatmap. The left part of the figure shows all genome interactions from both experiments, whereas the right part of the figure shows the strongest genome interactions for each experiment, filtered based on the 1% top-ranking *trans* interactions in experiment separately. In each part of the figure, the left horizontal line corresponds to chromosome 10, the right one to chromosome 11.

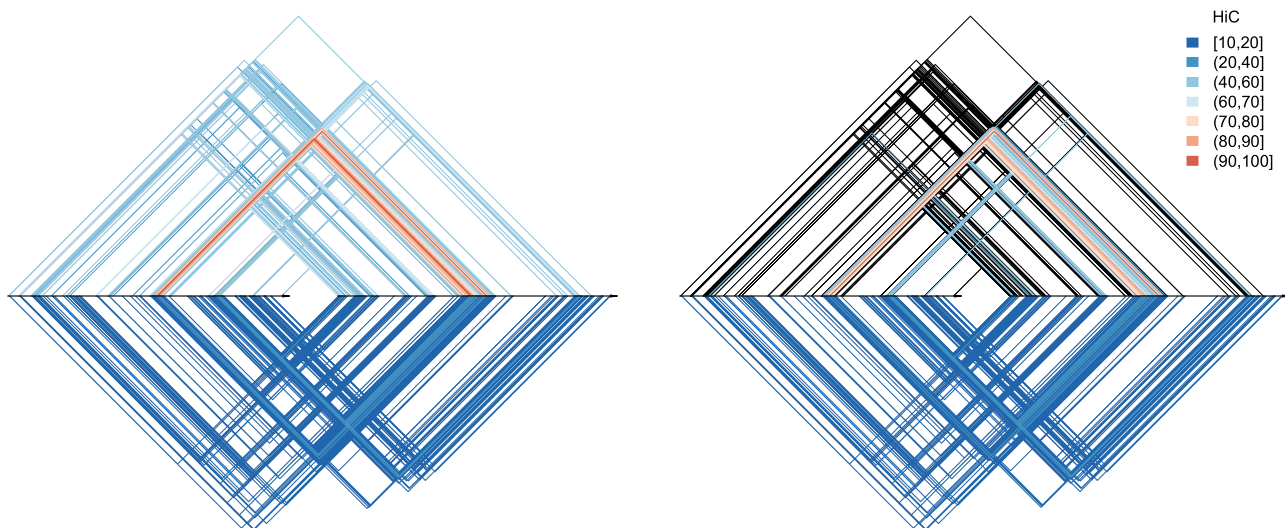

Figure 6. Plots showing the two experimental Hi-C data sets as in the right part of Figure 5. The left part of the figure here is identical to the right part of Figure 5, but now depicts the strongest interactions in terms of arcs linking the two interacting genome segments rather than a heatmap. The right part of the figure here shows the same interactions in terms of a comparison plot, where common genome interactions are shown in non-black on top (the color of each non-black arc reflects the maximum interaction strength of the genome interactions from both experiments), genome interactions that are only part of experiment 1 are shown in black on top, and genome interactions that are only part of experiment 2 are shown below.

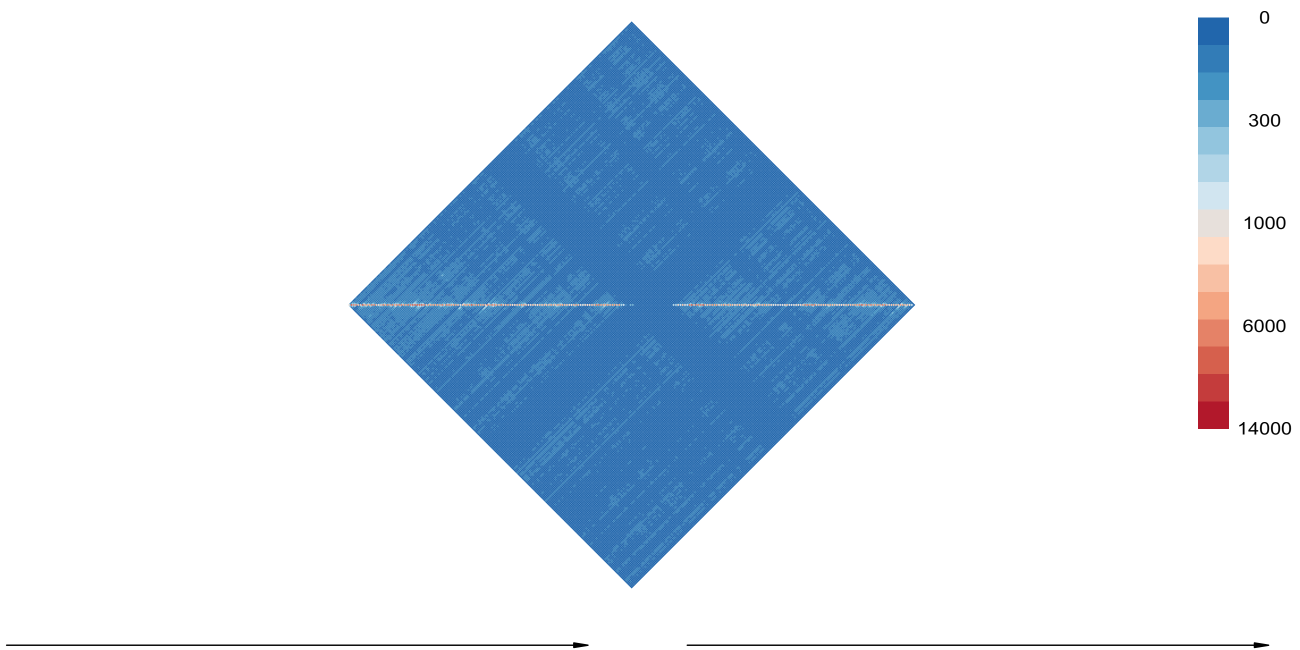

Figure 7. Figure showing the interactions between the chromosomal DNA and its expressed RNA for human chromosome 1 (35). A high reactivity (see the legend on the plot) can be observed for interacting segments (horizontal line). A base amount of interactions with a much lower reactivity can be observed for all of chromosome 1 (large area coloured in shades of blue). In addition, a large segment of zero reactivity can be observed (bands in dark blue) which corresponds to the 1q12 band region of chromosome 1.

re-wrote the original R4RNA R package underlying R-CHIE (1) and signficantly extended its functionality.

As R-CHIE figures can now involve multiple input entities (one or several RNA or DNA sequences; or one or multiple multiple-sequence alignments) and their interactions, it was necessarily to extend our original definition of the helix-format in which input information on *cis* and *trans* features is supplied. Please see the R4RNA manual and R-CHIE web server for the concise and extended helix-format definition. Existing users of R-CHIE can be reassured that the existing functions will continue to work with the helix-format definition so far. Information on the input sequences or their multiple-sequence alignments continues to be specified in terms of a fasta input file whose headers link information on the sequences to information on the respective *cis* and *trans* features in the helix input file.

The output of R-CHIE corresponds to a file in png- or pdf-format for each figure. The web server generates this out-

Table 1. Comparison of key features of R-CHIE and existing visualization packages. *Trans* RNA–RNA interactions are interactions involving base-pairs between two RNA transcripts. R-CHIE is the only tool capable of visualizing *trans* RNA–RNA interactions involving more than two transcripts (multiple entities) and of showing a multiple-sequence alignment (MSA) alongside information on RNA structure and *trans* RNA–RNA interaction features (MSA plot). Also, R-CHIE can explicitly highlight the extent of covariance within an MSA underlying RNA structure and *trans* RNA–RNA interaction features (covariance) which is key to quickly assessing the extend (or lack of) significant evolutionary support for individual *cis* or *trans* base-pairs. Another useful feature in that regard, which is supported both by RILOGO and R-CHIE, is the ability to compare two multiple-sequence alignments for the same RNA structure or *trans* RNA–RNA interaction features (compare two MSAs). Information on genomic interactions such as those obtained via Hi-C experiments or GAM can also be visualized with R-CHIE now (*cis DNA–DNA* and *trans* DNA–DNA interactions), including the visualization of quantitative information on the respective interaction strength (interaction strength). One key unique feature of R-CHIE are comparison plots (comparison plot) for which the communalities and differences between two alternative sets of features (e.g. predicted features versus reference features or experimental condition 1 versus experimental condition 2) are first calculated and then visualized. Of all the tools listed, R-CHIE is unique in that it combines the ability to both visualize RNA structure (*cis RNA–RNA* interactions) and *trans* RNA–RNA interaction features (which usually comprises nucleotide-specific information) as well as information on *cis* and *trans RNA–DNA* and *DNA–DNA* interactions, which is often more coarse-grained involving intervals of several kilobases

Tool	*trans* RNA–RNA interactions	*cis RNA–RNA* interactions	*trans* DNA–DNA interactions	*cis DNA–DNA* interactions	*trans* RNA–DNA interactions	Multiple entities	MSA plot	compare two MSAs	covariance	Interaction strength	comparison plot
RILOGO (28)	✓	✓	—	—	—	—	—	✓	—	—	—
MARIO TOOLS (29)	✓	✓	—	—	—	✓	—	—	—	—	—
3D GENOME BROWSER(30)	—	—	✓	✓	—	—	—	—	—	✓	—
GITAR (31)	—	✓	—	—	—	—	—	—	—	✓	—
GenomicInteractions (32)	—	—	✓	✓	—	—	—	—	—	✓	—
RNAFDL(33)	—	✓	—	—	—	—	—	—	—	—	—
VARNA(34)	—	✓	—	—	—	—	—	—	—	✓	✓
R-CHIE	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

put on the fly, typically requiring on the order of seconds to produce the figure. For every request, the web server displays the corresponding command-line underlying the generation of the figure. Novice users of R-CHIE can thus start with the examples provided for the different kinds of plots on the web-server and then explore additional features. In addition to these examples, the web server now also features mini-tutorials that can be readily explored online. For using R-CHIE on a local computer, the user needs to install the R4RNA R package as well as R itself on a local computer (R is freely downloadable from http://r-project.org/ for all major operating systems). Once locally available, the generation of figures with R-CHIE can be seamlessly integrated into existing computational pipelines for analysing data sets.

RESULTS AND DISCUSSION

We here present a new computational method for visualizing *cis* and *trans* RNA–RNA, *RNA–DNA* as well as *DNA–DNA* interactions. For this, we have significantly extended and technically completely revised an earlier version of R-CHIE that was initially published as a tool and web-server for visualizing RNA secondary structure information. The new version of R-CHIE offers a multitude of novel and unique types of figures that allow users to explore and understand their data in a more intuitive manner than via a purely numerical data analysis. This is primarily due to R-CHIE's ability to visualize key functional features such as RNA structure features, *trans* RNA–RNA, *cis* and *trans* RNA–DNA as well as *DNA–DNA* interactions alongside corresponding colour-coded quantitative information. This information can, for example, correspond to theoretically estimated *P*-values, experimentally derived interaction strengths or different types of evolutionary support and can readily be adapted to each user's particular types of evidence and visualization needs. Particularly noteworthy are the so-called comparison plots in R-CHIE that allow users to (first automatically compute and then directly) visualize the communalities and differences between two alternative sets of features, such as predicted features versus reference features or features that have been derived from two different experimental conditions. R-CHIE is also the most flexible tool when it comes to visualizing *trans* features involving more than two interacting entities, whether they be interacting RNA transcripts or interacting chromosomes or *trans* RNA–DNA interactions.

R-CHIE is readily accessible via our web-server at https://www.e-rna.org/r-chie and the corresponding R package R4RNA that can be downloaded from our web-server. We welcome suggestions and feedback from the research community. We intend to officially submit the completely revised R4RNA package to the BIOCONDUCTOR (20) repository in near future.

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